

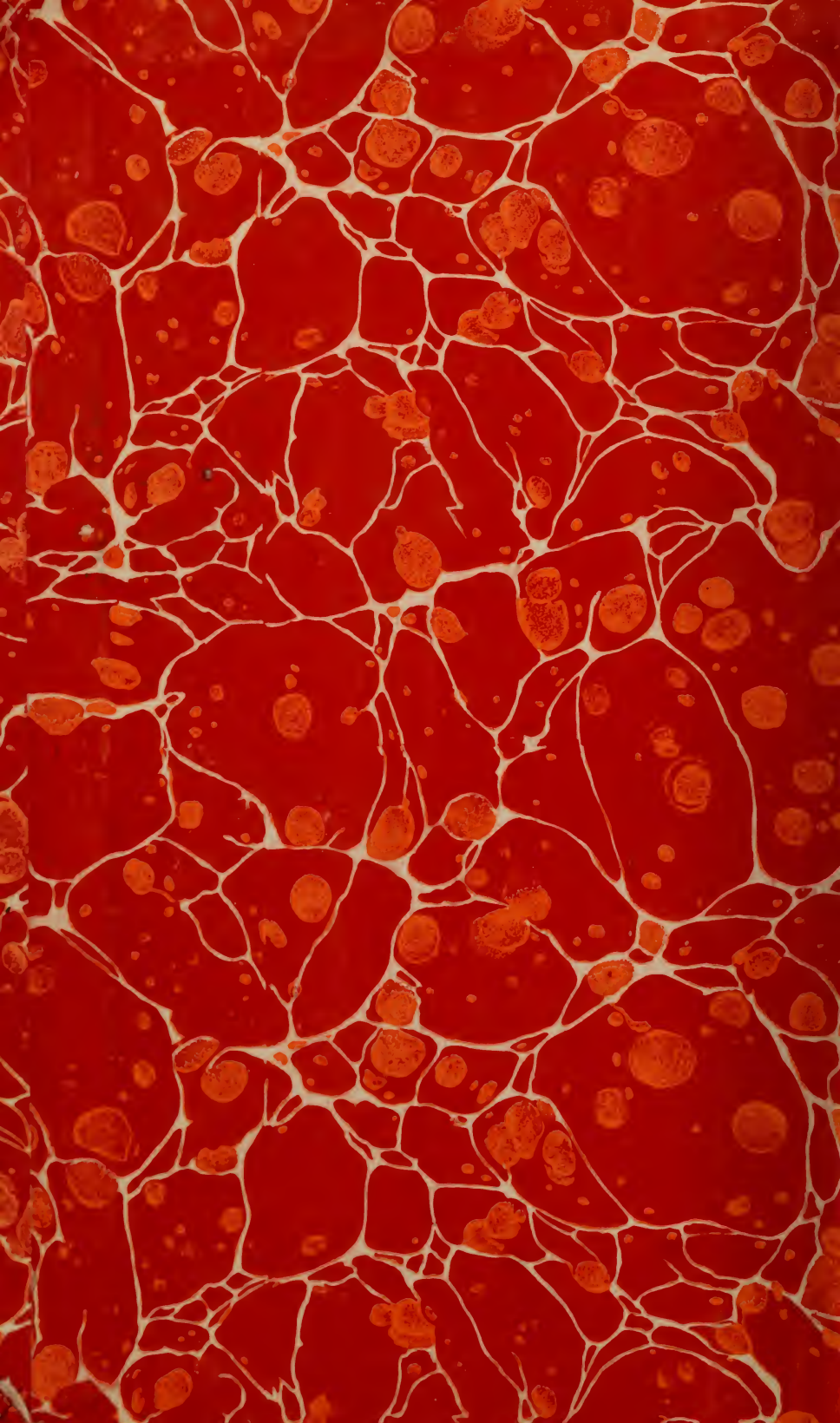
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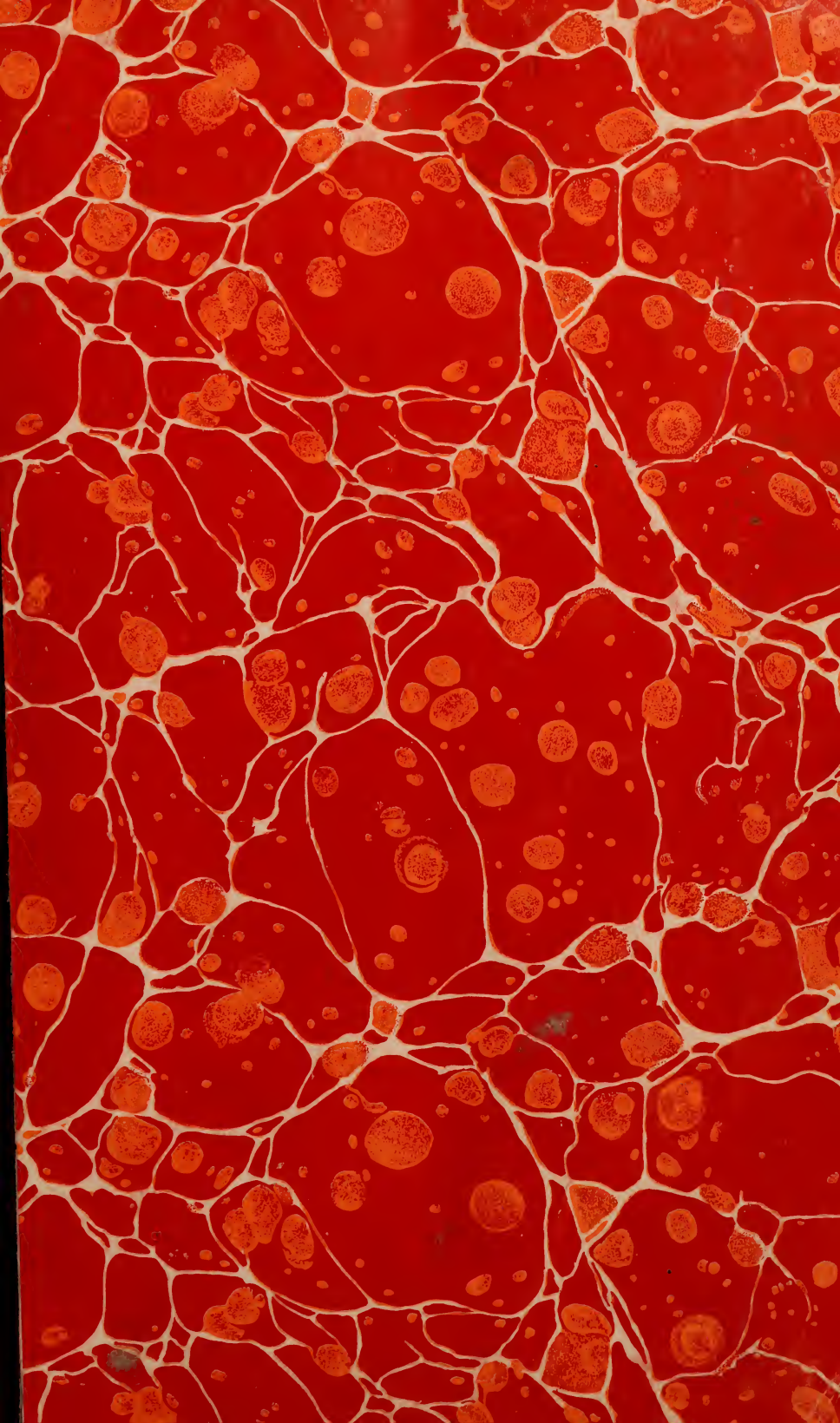


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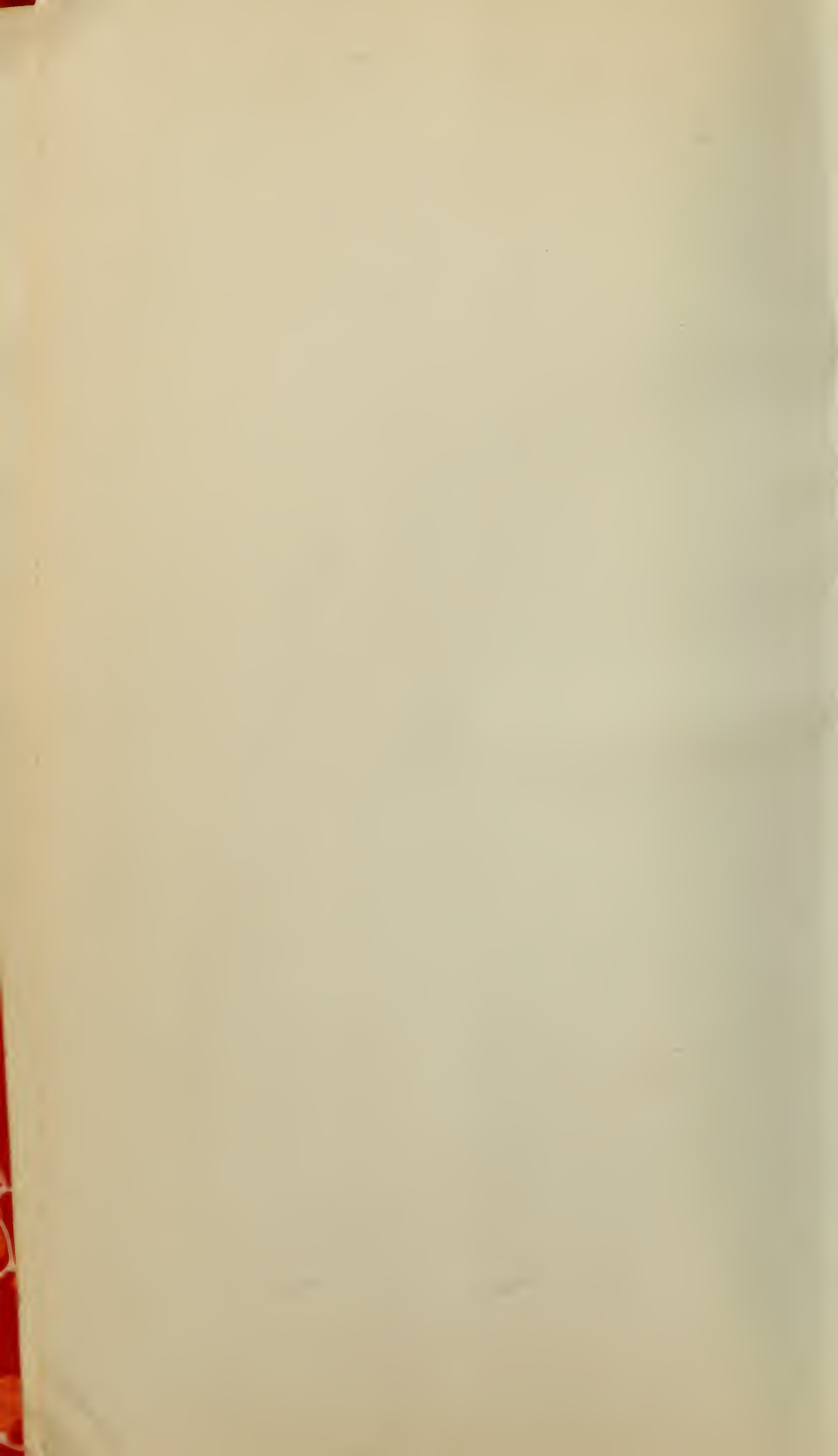


















# THE OXIDATION OF ALPHA AND BETA GLUCOSE AND A STUDY OF THE ISOMERIC FORMS OF THE SUGAR IN SOLUTION <sup>1</sup>

By Horace S. Isbell and Ward Pigman

## ABSTRACT

The method of bromine oxidation in the presence of barium carbonate has been applied to the measurement of the rates at which the various forms of *d*-glucose react with bromine water. Under the conditions described  $\beta$ -*d*-glucose is oxidized about thirty-five times as fast as  $\alpha$ -*d*-glucose. The delta lactone of gluconic acid is the predominating product in both cases, and it is formed without the intermediate production of gluconic acid. This indicates that  $\alpha$ - and  $\beta$ -*d*-glucose contain the 1, 5 or amylenoxide ring. The oxidation of an equilibrium solution of *d*-glucose progresses rapidly, until all of the beta glucose present in the solution is used up, and then more slowly as the oxidation of the alpha form is continued. The rate of oxidation indicates that the equilibrium solution contains about 64 per cent beta and 36 per cent alpha glucose. There can be little doubt but that the equilibrium of glucose is more complex than the simple equilibrium between the alpha and beta forms, but the absence of substantial amounts of the free acid and the gamma lactone in the product of oxidation is evidence that the equilibrium solution of the sugar does not contain large proportions of the free aldehyde and of the gamma form. It is believed that the application of the methods of investigation described in this report will yield much useful information as to the composition of sugar solutions and the changes which occur during the mutarotation reaction.

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## I. INTRODUCTION

Inasmuch as solutions of reducing sugars presumably contain a number of isomeric forms of the sugar in equilibrium, it is desirable to ascertain which isomer is the active reductant in any oxidation. Previous investigators have assumed that solutions of the aldoses contain small quantities of the aldehyde form of the sugars and that the monocarboxylic acid is derived by the oxidation of this free aldehyde. It was shown by Isbell and Hudson <sup>2</sup> that the optical rotation of a buffered glucose solution, upon bromine oxidation, rises to a point which corresponds with the rotation of the delta lactone, and then the rotation decreases to a minimum value at a rate approximately

<sup>1</sup> This paper was presented at the meeting of the American Chemical Society, New Orleans, La., March 29, 1932.

<sup>2</sup> Isbell and Hudson, B. S. Jour. Research, vol. 8 (RP418), p. 327, 1932.

the same as the hydrolysis of the delta lactone to the corresponding acid. Subsequently it was shown by Isbell<sup>3</sup> that the free acid is not formed in the course of the oxidation of the aldoses with bromine water by conducting the reaction in the presence of barium carbonate. The latter neutralizes the free acid but does not react with the lactone, thereby differentiating between the two substances. This method, as well as the changes in optical rotation and acidity, prove that delta lactones are obtained prior to the acids from  $\alpha$ - and  $\beta$ -*D*-mannose,  $\alpha$ - and  $\beta$ -*L*-rhamnose,  $\alpha$ - and  $\beta$ -lactose, and  $\beta$ -maltose. Therefore, it is apparent that the normal aldoses, at least in slightly acid solution, do not yield acids by bromine oxidation and consequently there is no foundation for the heretofore accepted concept that the reaction takes place through the straight-chain aldehyde form of the sugar. Since, in strongly acid solutions, the oxidations are slow and the oxidation products are rapidly converted into equilibrium mixtures containing the free acid, the delta and gamma lactones, extreme care must be exercised in the interpretation of the results thus obtained.

In a paper entitled "A Possible Source of Error in Determining the Constitution of Di and Polysaccharides," published prior to the publication of Isbell and Hudson,<sup>4</sup> on "The Course of the Oxidation of the Aldose Sugars with Bromine Water," but subsequent to the actual work done by Isbell and Hudson, Irvine and McGlynn<sup>5</sup> make the following statement: "Crystalline 2, 3, 6 trimethyl glucose if oxidized with bromine water under conditions which permit the accumulation of hydrogen bromide yields directly the gamma lactone of 2, 3, 6 trimethyl gluconic acid as the main product—but when the sugar is oxidized by the same reagent in the absence of halogen acid, the delta lactone is produced in preponderating amount." However, as pointed out by Hirst,<sup>6</sup> their experimental work does not show that the gamma lactone is formed directly because the reaction in acid solution was allowed to continue eight hours, which is ample time, especially in the presence of hydrobromic acid, for the primary oxidation product to be rearranged into the gamma lactone. Irvine and McGlynn's experimental procedure, also, did not show that the delta lactone was the primary oxidation product of the bromine oxidation in the absence of halogen acid, because they allowed the reaction to proceed for eight hours in the presence of calcium carbonate. In eight hours the lactone would have been hydrolyzed, for the most part, to give the free acid, which would have reacted in turn with calcium carbonate to give the calcium salt. Irvine and McGlynn state: "The calcium salts in solution were decomposed by the addition of the required amount of oxalic acid, the product extracted with chloroform and isolated in the usual manner." By this procedure a mixture containing the delta and gamma lactones would have been obtained regardless of the nature of the initial oxidation product.

## II. OXIDATION OF ALPHA AND BETA GLUCOSE

The method, which has been reported in previous publications,<sup>7</sup> consists in oxidizing the sugar with bromine water in a solution saturated with carbon dioxide and buffered with a suspension of barium

<sup>3</sup> Isbell, B. S. Jour. Research, vol. 8 (RP441), p. 615, 1932.

<sup>4</sup> See footnote 2, p. 337.

<sup>5</sup> Irvine and McGlynn, J. Am. Chem. Soc., vol. 54, p. 359, 1932.

<sup>6</sup> Hirst, J. Am. Chem. Soc., vol. 54, p. 2559, 1932.

<sup>7</sup> Isbell, see footnote 3, p. 338; also J. Am. Chem. Soc., vol. 54, p. 1692, 1932.

carbonate. The barium carbonate prevents the solution from becoming strongly acid, and consequently the oxidation is rapid, while the mutarotation reaction is slow. In the present instance the crystalline sugar or the equilibrium mixture was added to the buffered bromine solution and from time to time the oxidation was stopped by removing the bromine through reaction with an unsaturated oil or hydrocarbon; the excess buffer (barium carbonate) was then separated by filtration, leaving a solution containing the oxidation product, any unoxidized sugar, barium bromide, and traces of barium bicarbonate. Any free gluconic acid, formed either in the course of the oxidation or by the hydrolysis of the lactone, would be neutralized by the barium carbonate giving barium gluconate, while the unchanged lactone would exist in the solution as such. The amount of the lactone in the solution was determined by titration; the unoxidized sugar was determined by reduction of Benedict's solution; and the amount of gluconic acid neutralized by the barium carbonate was estimated by difference. Portions of the solutions containing the oxidation products were used also for measuring the optical rotations and the hydrogen-ion concentrations. The results obtained from  $\alpha$  and  $\beta$ -*d* glucose and the equilibrium solution are given in Tables 1 and 2.

TABLE 1.—Comparison of the oxidation products with the delta lactone

$\alpha$ - <i>d</i> -glucose, oxidation 85.5 per cent complete in 20 minutes			$\beta$ - <i>d</i> -glucose, oxidation 97.5 per cent complete in 3 minutes			<i>d</i> -glucose in equilibrium, oxidation 77.2 per cent complete in 5 minutes			Gluconic delta lactone		
Minutes	$[\alpha]_D^{20}$	pH <sup>1</sup>	Minutes	$[\alpha]_D^{20}$	pH	Minutes	$[\alpha]_D^{20}$	pH	Minutes	$[\alpha]_D^{20}$	pH
26	50.6	5.1	13	62.4	4.8	14	65.8	4.9	13	61.7	4.6
30	49.7	4.5	15	61.9	4.5	15	65.6	4.7	15	61.4	4.3
40	47.8	3.9	25	60.1	3.6	30	61.5	3.7	25	59.9	3.5
90	39.8	3.3	40	57.3	3.2	45	58.0	3.6	40	57.1	3.1
210	26.8	3.0	60	52.0	2.9	73	52.4	3.3	60	53.8	2.8
355	18.0	2.9	150	27.8	2.5	133	42.3	3.0	150	32.0	2.4
Hours			Hours			Hours			Hours		
24	12.0	-----	305	10.1	2.5	338	24.1	2.7	315	14.6	2.4
			24	7.1	-----	22	19.4	-----	24	7.5	-----

<sup>1</sup> Measurements made at 25° C. in solutions containing CO<sub>2</sub> and BaBr<sub>2</sub>.

TABLE 2.—Saponification of the oxidation products in solutions buffered with BaCO<sub>3</sub> and CO<sub>2</sub> at 25° C.<sup>1</sup>

Product from $\alpha$ - <i>d</i> -glucose		Product from $\beta$ - <i>d</i> -glucose		Product from equi- librium solution of <i>d</i> -glucose		Gluconic delta lactone	
Time	Per cent of product not saponi- fied	Time	Per cent of product not saponi- fied	Time	Per cent of product not saponi- fied	Time	Per cent of product not saponi- fied
Minutes		Minutes		Minutes		Minutes	
25	87.4	7	95.8	9	87.1	7.5	93.6
85	63.0	65	70.8	67	63.3	95	62.8
205	40.2	186	43.7	181	36.9	186	44.8
326	29.5	300	26.3	318	21.5	300	20.65
1,440	10.7	1,440	0.7	1,440	2.85	1,440	00

<sup>1</sup> The measurements were made with portions of the solutions given in Table 1.



The sample of  $\beta$ -*d*-glucose was oxidized to the extent of 97.5 per cent in 3 minutes at 25° C., and 7 minutes after the beginning of the oxidation 95.8 per cent of the product existed in the solution as unchanged lactone. Inasmuch as a sample of the delta lactone did not give a higher percentage of unchanged lactone after shaking 7 minutes with barium carbonate, the small fraction (4.2 per cent) of the oxidation product which was apparently neutralized by the barium carbonate probably resulted from the acid set free by hydrolysis. Hence there is very little, if any, free gluconic acid formed by the bromine oxidation of  $\beta$ -*d*-glucose in the presence of barium carbonate. The solution containing the lactone or oxidation product from  $\beta$ -*d*-glucose gave  $[\alpha]_D^{20} = +62.4^\circ$ , 13 minutes after the addition of the bromine, and in 24 hours the specific rotation decreased to a minimum value of  $+7.1$  and then slowly increased. The delta lactone of gluconic acid under similar conditions gave  $[\alpha]_D^{20} = +61.7^\circ$ , 13 minutes after solution, decreasing in 24 hours to a minimum of  $+7.5$  and thereafter slowly increasing in value. These results are in qualitative agreement with the work of Nef<sup>8</sup> on the delta lactone, who also reported that the gamma lactone, on the other hand, gives  $[\alpha]_D^{20} = +67.82$  initially, which decreases in 24 hours to  $[\alpha]_D^{20} = +61.3$ .

The changes in the hydrogen-ion concentration (pH) of the products obtained from  $\alpha$ - and  $\beta$ -*d*-glucose, as well as the equilibrium solution, show that the products obtained after removing the barium carbonate develop acidity on standing in aqueous solution. The changes in acidity on standing are similar to those observed for the delta lactone of gluconic acid under like conditions.

The rates of saponification given in Table 2 were determined by titration after shaking the samples with a suspension of barium carbonate in water, saturated with carbon dioxide. The curves in Figure 1 were obtained by plotting the data on a semilogarithmic scale. The amount of unchanged lactone is expressed as the logarithm of that fraction of the product, originally added or theoretically formed by oxidation, which remains at any given time. Since the slopes of the curves are nearly the same the various products are saponified at approximately equal rates.

From Table 2 it can be seen that after 186 minutes, 43.7 per cent of the oxidation product from  $\beta$ -*d*-glucose had not reacted with barium carbonate and that under like conditions gluconic delta lactone was unchanged to the extent of 44.8 per cent. After 24 hours no unchanged gluconic delta lactone remained in the solution, while under like conditions 0.7 per cent of the oxidation product derived from  $\beta$ -*d*-glucose remained in a form capable of neutralizing an alkali.

The oxidation of  $\alpha$ -*d*-glucose probably takes place in much the same manner as the oxidation of  $\beta$ -*d*-glucose, but since the alpha form is oxidized more slowly than the beta form, the measurement of the properties of the oxidation product derived from  $\alpha$ -*d*-glucose is complicated by the presence of unoxidized sugar and by side reactions which may occur during the relatively long time required for the reaction. The changes in rotation, hydrogen-ion concentration, and the rates of saponification show qualitatively that the delta lactone is the principal product derived by the oxidation of  $\alpha$ -*d*-glucose with bromine water in the presence of barium carbonate. But as shown

in Table 2, 10.7 per cent of the oxidation product derived from alpha glucose and 2.8 per cent of the product derived from the equilibrium solution were not saponified in 24 hours (1,440 minutes). This relatively inert material may arise from some form of the sugar which is produced during the mutarotation reaction. The oxidation of the alpha sugar required a longer time and consequently there was more opportunity for the oxidation of substances which might be formed in the course of the mutarotation reaction. However, the oxidation of a solution of *d*-glucose in equilibrium does not reveal the presence of a large quantity of products other than the delta lactone. The oxidation of the equilibrium solution of *d*-glucose, comparable to the oxidations of alpha and beta sugars which have been given, was 77.2 per cent complete in 5 minutes, and 4 minutes later 87.1 per cent of the

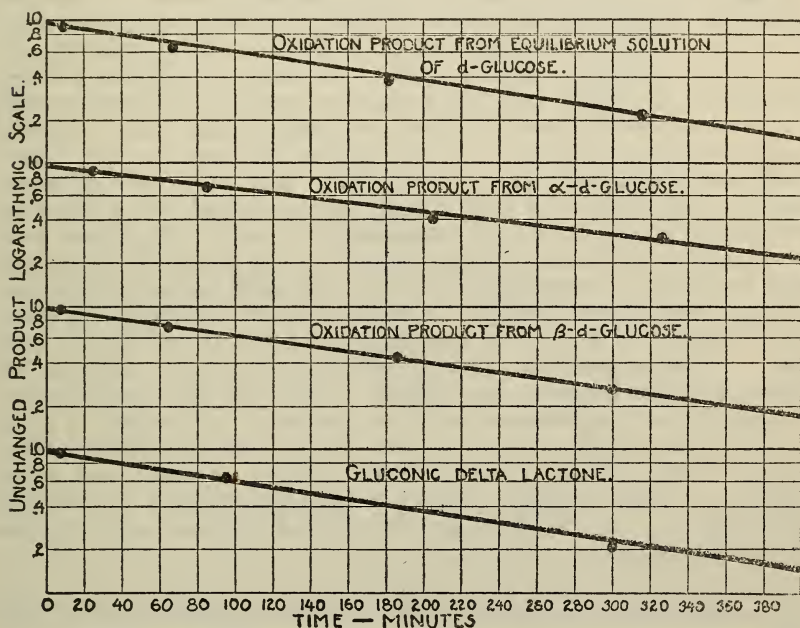


FIGURE 1.—Saponification in solutions buffered with  $\text{BaCO}_3$  and  $\text{CO}_2$

reaction product remained in the solution as unchanged lactone. As noted above, this unchanged lactone contains a small amount of some substance which is saponified more slowly than the delta lactone. Inasmuch as this substance may give valuable information in regard to intermediate products of the mutarotation reaction, it is being investigated further in order to ascertain what it is and to determine the conditions which give rise to its formation.

As a final step in the proof for the formation of the delta lactone by oxidation of  $\beta$ -*d*-glucose, the crystalline delta lactone was separated from the reaction mixture. The oxidation was conducted in the same manner as before, but the product, which contained very little unoxidized sugar, was extracted with a solvent (dioxane) which dissolves the lactone, but does not dissolve the inorganic salts. The solution of the oxidation product, on evaporation at a low temperature in



vacuo, readily gave the crystalline delta lactone.<sup>9</sup> In some cases the delta lactone was separated within one-half hour after the addition of the sugar to the bromine water, the experimental conditions being such that an alteration in the oxidation product was unlikely.

The physical and chemical measurements, which have been given, prove that the delta lactone is the predominating product of the reaction in each case and that it does not originate from gluconic acid by spontaneous lactone formation. It is emphasized that the measurements were made during the course of the oxidation and immediately thereafter, thereby confirming the previously advanced hypothesis of Isbell and Hudson that the cyclic forms of the normal sugars give delta lactones by oxidation with bromine water.

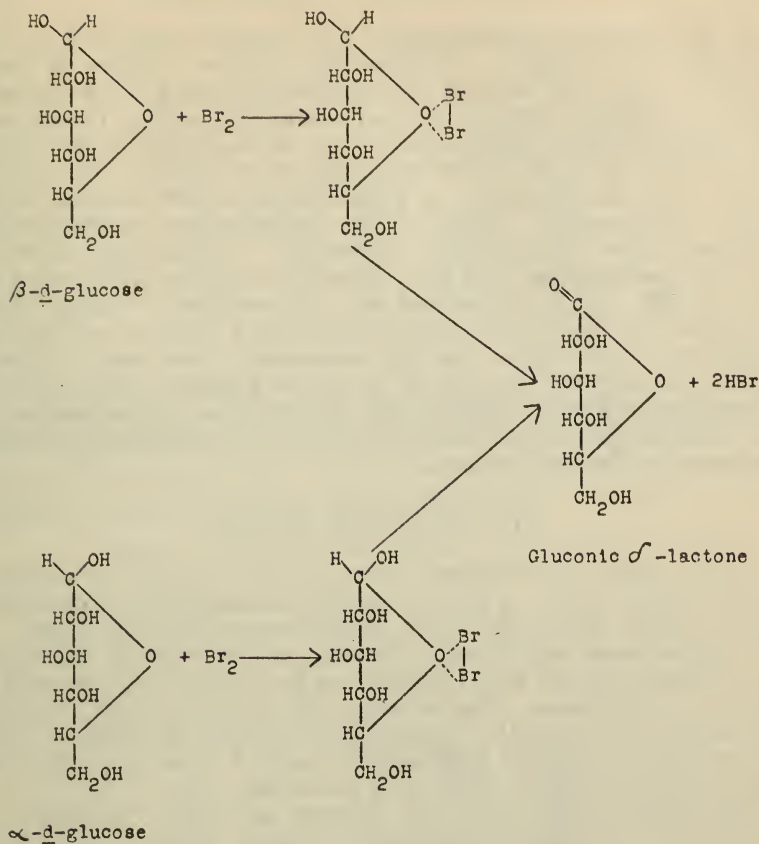
At this time there is no direct experimental evidence as to the exact mechanism of the transformation of the cyclic form of the sugar to the lactone. The consideration of the subject is of importance in so far as it will stimulate further work. As shown in Section III the oxidations of the alpha and beta sugars are rapid in comparison with the mutarotation reaction; hence we can conclude that only a limited amount of interconversion can take place between the two forms during the oxidations. But it is possible that prior to the actual oxidation step, alpha and beta glucose are converted into a common intermediate product, such as Armstrong's<sup>10</sup> enolic form of the oxonium hydrate. However, a consideration of the velocities of the oxidation reactions in comparison with the rate of mutarotation makes this hypothesis appear unlikely. Any satisfactory interpretation must explain the large difference in the reaction rates of the alpha and beta forms. It seems probable that the reaction takes place through an addition product between the oxygen of the ring and the bromine. This concept is in agreement with the tendency of bromine to form molecular compounds and it is also in accord with the ability of the sugars to form addition products with calcium chloride and other substances, a characteristic which has been emphasized in previous publications.<sup>11</sup> The reactions are represented hypothetically by the following equations:

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<sup>9</sup> Since the delta lactones are obtained from the aldoses in general, the oxidation method is being applied to the preparation of some of the delta lactones of aldonic acids which have not been obtained by other methods.

<sup>10</sup> Armstrong, *The Carbohydrates and the Glucosides*, p. 47, Longman's, Green & Co., 1924.

<sup>11</sup> Isbell, B. S. *Jour. Research*, vol. 5 (RP226), p. 741, 1930; vol. 7 (RP396), p. 1, 1931.



If a bromine addition product is formed as illustrated above, when the hydrogen of carbon one is on the same side of the ring and in the vicinity of the bromine it will be in a favorable position for oxidation. When the hydrogen is on the opposite side of the ring it will be in a less favorable position for oxidation and, since the hydrogen of the hydroxyl group, which is now in proximity to the bromine, is already in an oxidized state, the reaction will proceed more slowly. In models of  $\alpha$ - and  $\beta$ -d-glucose with the hydrogen and hydroxyls on the first carbon allocated according to the work of Boëseken<sup>12</sup> the hydrogen on the first carbon lies on the same side of the ring as the oxygen in beta glucose and on the opposite side in alpha glucose. Hence the hydrogen is in a favorable position for oxidation in the beta form and in a less favorable position in the alpha. The spacial relations may be best understood by recourse to the conventional 3-dimensional models of alpha and beta glucose which can be conveniently made by means of tetrahedrons such as those described by Brauns.<sup>13</sup>

Inasmuch as there are several interpretations which may be advanced to account for the changes in the reaction rates with changes

<sup>12</sup> Boëseken, Ber., vol. 46, p. 2612, 1913.

<sup>13</sup> Brauns, D. H., J. Am. Chem. Soc., vol. 51, p. 1824, 1929.

in the hydrogen-ion concentration, more experimental data must be obtained on this subject. As shown by the work of Lobry de Bruyn, Nef, and others, the sugar in the presence of strong alkali undergoes profound changes, while in neutral solution a portion of the sugar dissociates to form a negative ion which, as postulated by Bunzel and Mathews,<sup>14</sup> may react at a different rate from the other forms of the sugar. Also, the character of the oxidizing agent changes as neutrality is approached. Thus, as the hydrogen-ion concentration is decreased, more hypobromite is present. It has been shown by previous investigators that the aldoses in alkaline solution are oxidized rapidly by hypobromites and hypiodites.<sup>15</sup> Under certain conditions the open-chain form of the sugar may be oxidized and perhaps this is the reason that the oxidation is very rapid in alkaline solution. Since the method as used for studying the reaction is restricted to a limited range in the hydrogen-ion concentration, the rôle of the hydrogen and hydroxyl ions in the reaction will not be considered in this publication.

### III. REACTION RATES

In 1925 Kuhn and Wagner-Jauregg<sup>16</sup> found that the beta forms of certain sugars reduce a given amount of potassium permanganate about 1.7 times as rapidly as the alpha forms of the same sugars. In their experiments only a small fraction of the sugar in the sample was oxidized, and the time required was large in comparison with the amount of oxidation. Furthermore, the reaction with potassium permanganate is complex, leading to a number of products, which indicates that the sugar molecule is attacked at several points and consequently the results are largely empirical.

The bromine oxidation in the presence of barium carbonate was selected for the present investigation because it yields nearly quantitatively a single product and may be studied kinetically to obtain valuable information as to the substances involved in the reaction. Particular attention was directed to maintaining conditions such that there was very little alteration in the sugar prior to oxidation. The experimental method was essentially as outlined on page 339 except that the temperature was maintained at 0° C. and samples were taken at more frequent intervals. Under these conditions, as previously noted by Isbell,<sup>17</sup> the beta sugars are oxidized much more rapidly than the alpha sugars. Our results show that beta glucose is oxidized about 35 times as rapidly as alpha glucose, a much larger difference than found by the method of Kuhn and Wagner-Jauregg.

<sup>14</sup> Bunzel and Mathews, *J. Am. Chem. Soc.*, vol. 31, p. 464, 1909.

<sup>15</sup> Romijn, Z., *Anal. Chem.*, vol. 36, p. 349, 1897; also Goebel, *J. Biol. Chem.*, vol. 72, p. 802, 1927.

<sup>16</sup> Kuhn and Wagner-Jauregg, *Ber.*, vol. 58, p. 1441, 1925.

<sup>17</sup> See footnote 3, p. 338.



TABLE 3.—Oxidation of  $\beta$ -*d*-glucose<sup>1</sup> at  $0.3^{\circ} \text{ C.} \pm 0.3^{\circ}$ 

Time	Unoxi- dized sugar	Oxidized sugar	$ak_{\beta}$ <sup>2</sup>	$k_{\beta}$
1	2	3	4	5
<i>Minutes</i>	<i>g</i>	<i>Per cent</i>		
1.3	3.495	22.4		
3.3	2.330	48.2	0.0878	
5.1	1.655	63.2	.0853	
8.3	.837	81.4	.0886	
11.1	.483	89.3	.0878	
15.5	.204	95.5	.0871	
25.3	.080	98.2	(.0681)	
		Average.	.0873	1.26

<sup>1</sup> Crystalline  $\beta$ -*d*-glucose (4.5 g) was added to a mixture of 500 ml of aqueous barium bromide, 10 ml of bromine, and 30 g of barium carbonate. The barium bromide solution contained 30 g of  $\text{BaBr}_2 \cdot 2\text{H}_2\text{O}$  and was saturated with  $\text{CO}_2$ . The average of the bromine determinations by  $\text{Na}_2\text{S}_2\text{O}_3$  titrations was 0.351 moles per liter and the average bromide content (calculated as given on p. 355) was 0.412 moles per liter. The concentration of free bromine as determined from equation (8), p. 348, was 0.0692 moles  $\text{Br}_2$  per liter.

<sup>2</sup>  $ak_{\beta} = \frac{1}{t} \log \frac{A}{A-x}$ , where  $A$  is the amount of sugar (77.6 per cent) at the time of the first observation, and  $x$  is the amount of oxidation during the time,  $t$ , which is measured from the first observation. The concentration of the oxidant (free bromine) is represented by  $a$ . (See p. 346.)

TABLE 4.—Oxidation of  $\alpha$ -*d*-glucose at  $0.3^{\circ} \text{ C.} \pm 0.3^{\circ}$  <sup>1</sup>

Time	Unoxi- dized sugar	Oxidized sugar	Bromine by $\text{Na}_2\text{S}_2\text{O}_3$ titration	$k_1 + ak_{\alpha}$ <sup>2</sup>	$ak_{\alpha}$	$k_{\alpha}$
1	2	3	4	5	6	7
<i>Minutes</i>	<i>g</i>	<i>Per cent</i>	<i>Moles per l</i>			
1.4	4.59	0	0.363			
5.1	4.47	2.6	.372	(0.00313)	(0.00261)	
30.0	3.83	16.6	.374	.00276	.00224	
60.1	3.27	28.8	.378	.00252	.00200	
120.0	2.335	49.1	.374	.00247	.00195	
180.2	1.640	64.3	.371	.00250	.00198	
240.0	1.190	74.1	.367	.00246	.00194	
300.2	0.565	87.7	.396	(.00305)	(.00253)	
Average			.374	.00254	.00202	0.0238

<sup>1</sup> The sample of  $\alpha$ -*d*-glucose was treated like the sample of beta glucose given in footnote 1, Table 3. The average bromide content (calculated as given on p. 355) was 0.381 moles per liter, and the average concentration of free bromine as calculated by equation (8), p. 348, was 0.085 moles of  $\text{Br}_2$  per liter.

<sup>2</sup>  $k_1 + ak_{\alpha} = \frac{1}{t} \log \frac{A}{A-x}$ , where  $A$  is the amount of sugar at the beginning of the time interval,  $t$ , and  $x$  is the amount of oxidation. The value  $k_1$  is assigned a value of 0.00052. (See p. 349.)

TABLE 5.—Oxidation of *d*-glucose equilibrium solution at  $0.5^{\circ} \text{ C.} \pm 0.3^{\circ}$  <sup>1</sup>

Time	Unoxidized sugar	Oxidized sugar	Bromine by $\text{Na}_2\text{S}_2\text{O}_3$ titration
1	2	3	4
<i>Minutes</i>	<i>g</i>	<i>Per cent</i>	<i>Moles per l</i>
1.3	3.90	13.4	0.346
4.8	2.74	39.1	.340
9.7	1.99	55.8	.338
15.0	1.76	60.9	.336
30.2	1.56	65.3	.336
122.4	1.055	76.6	.329
239.9	.710	84.2	.322
329.9	.560	87.6	.312

<sup>1</sup> An equilibrium solution of *d*-glucose containing 4.5 g of sugar was added to a solution of bromine in aqueous barium bromide which gave a mixture similar to that described in footnote 1, Table 3. The average of the bromine determinations by  $\text{Na}_2\text{S}_2\text{O}_3$  titration was 0.332 moles per liter, the average bromide content was 0.412 moles per liter, and the average concentration of free bromine was 0.063 moles per liter.

As shown in Figure 2, the oxidation of the sample of beta glucose was 50 per cent complete in about 3 minutes, while under like conditions the alpha form required about 130 minutes. The oxidation of the equilibrium solution progressed rapidly until the beta sugar was used up and then more slowly as the alpha form continued to be oxidized. This marked change in the reaction rate, during the oxidation of the equilibrium solution, has not been noted by previous investigators. As will be shown in subsequent publications, the study of the reaction rates affords means for ascertaining the proportions of the alpha and beta forms of the sugar in aqueous solutions and for deriving information concerning the equilibria existing in such solutions.

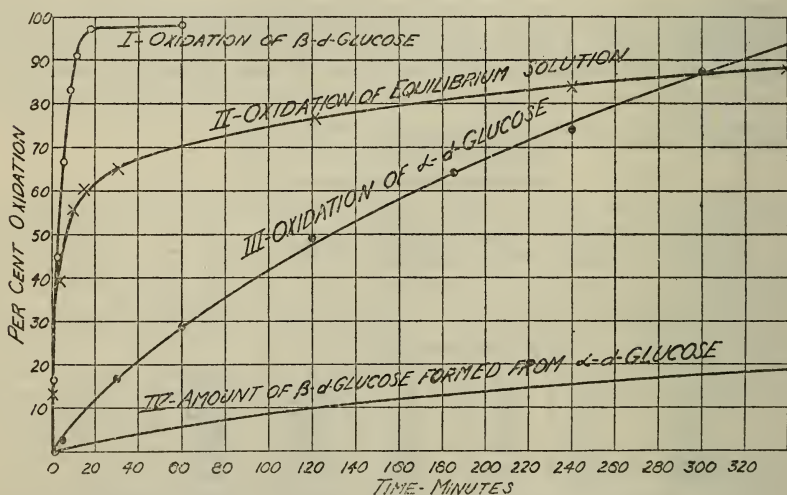


FIGURE 2.—Bromine oxidation of *d*-glucose in the presence of  $\text{BaCO}_3$

To simplify conditions, by making the reaction practically a monomolecular one, a large excess of bromine was used. Under such conditions the velocity constant for the reaction may be calculated by means of the formula:

$$ak_{\beta} = \frac{1}{t} \log \frac{A}{A-x}$$

where  $A$  is the amount of sugar at zero time,  $x$  is the quantity of sugar which has been oxidized during the time interval,  $t$ , and  $a$  is the concentration of the oxidant.

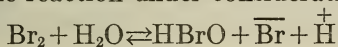
The data for the experiment on  $\beta$ -*d*-glucose, which is illustrated in Figure 2, are given in Table 3. The fairly uniform values of  $ak_{\beta}$ , given in column 4, show that the reaction proceeded at a uniform rate. This is evidence that the sample does not contain two forms of the sugar which are oxidized at different rates. That is,  $\beta$ -*d*-glucose  $[\alpha]_D^{20} = +19^{\circ}$ , is a homogeneous substance and not a mixture containing two isomers, such as the molecular compounds of lactose reported by Hockett and Hudson.<sup>18</sup>

<sup>18</sup> Hockett and Hudson, J. Am. Chem. Soc., vol. 53, p. 4455, 1931.



Since in the experiment under discussion the concentration of the oxidant was maintained practically constant, the effect of varying the concentration of bromine will now be considered.

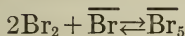
Previously Bunzel and Mathews<sup>19</sup> studied the rate of the oxidation of equilibrium solutions of *d*-glucose with bromine water. They found that the reaction was retarded by the presence of small quantities of acid and that at a given hydrogen-ion concentration the rate of the reaction depended on the concentration of the sugar and the free bromine. They used a large excess of sugar and measured the rate at which the bromine decreased in the solution. Inasmuch as only a fraction of the total sugar was oxidized, their results do not reveal the changes in the reaction rates reported in this paper. It is necessary to study the oxidation of the alpha and beta forms of the sugars separately in order to determine the true characteristics of the individual reactions. In the present investigation, measurements of the reaction rates of  $\alpha$  and  $\beta$ -*d*-glucose using different concentrations of bromine and barium bromide were made in order to determine whether the total bromine, free bromine, or hypobromous acid is the active oxidant. Since we were unable to find any data relating to the concentration of free bromine in barium bromide solutions, for our calculations, we used formulas derived by Jones and Hartmann<sup>20</sup> for bromine-potassium bromide solutions. The concentrations of free bromine and hypobromous acid thus obtained are only approximate and are applied to the problem at hand with that understanding. Jones and Hartmann assigned the following equilibrium constants to the reaction under consideration:



$$K_H = \frac{(\overset{+}{\text{H}})(\overline{\text{Br}})(\text{HBrO})}{(\text{Br}_2)} = 5.7 \times 10^{-10} \quad (1)$$



$$K_3 = \frac{(\text{Br})(\text{Br}_2)}{(\overline{\text{Br}}_3)} = 0.051 \quad (2)$$



$$K_5 = \frac{(\overline{\text{Br}})(\text{Br}_2)^2}{(\overline{\text{Br}}_5)} = 0.0246 \quad (3)$$

$$B = \overline{\text{Br}} + \overline{\text{Br}}_3 + \overline{\text{Br}}_5 \quad (4)$$

$$C = \text{Br}_2 + \overline{\text{Br}}_3 + 2\overline{\text{Br}}_5 + (\text{HBrO}) \quad (5)$$

The symbols represent the concentrations of the various ions and molecules in the equilibrium solution. The concentrations of the free bromine ( $\text{Br}_2$ ) and the hypobromous acid ( $\text{HBrO}$ ) are obtained by solving Jones and Hartmann's equations simultaneously and substituting the experimental values for the total bromine ( $C$ ), total bromide ( $B$ ), and hydrogen-ion concentration ( $\overset{+}{\text{H}}$ ). As the concentration of  $\text{HBrO}$  is extremely small, it was neglected in equation (5). Equation (6) is obtained by substituting in equation (4) the value of  $\overline{\text{Br}}_3$  derived

<sup>19</sup> Bunzel and Mathews, J. Am. Chem. Soc., vol. 31, p. 464, 1909; also Bunzel, J. Biol. Chem., vol. 7, p. 157, 1910.

<sup>20</sup> Jones and Hartman, Trans. Am. Electrochem. Soc., vol. 30, p. 295, 1916.

from equation (2) and the value of  $\overline{\text{Br}}_5$  derived from equation (3) and then solving for  $\overline{\text{Br}}$ .

$$\overline{\text{Br}} = \frac{B}{1 + \frac{\text{Br}_2}{0.051} + \frac{\text{Br}_2^2}{0.0246}} \quad (6)$$

Equation (7) is obtained by eliminating  $\overline{\text{Br}}_5$  between equations (4) and (5).

$$2B - C = 2\overline{\text{Br}} - \text{Br}_2 + \overline{\text{Br}}_3 \quad (7)$$

By substituting in equation (7) values of  $\overline{\text{Br}}_3$  and  $\overline{\text{Br}}$  in terms of  $\text{Br}_2$  as derived from equations (2) and (6), respectively, and simplifying, equation (8) is obtained. This equation was used for determining the concentration of free bromine.

$$\text{Br}_2^3 + (0.482 + 2B - C)\text{Br}_2^2 + [0.0246 + 0.482(B - C)]\text{Br}_2 - 0.0246C = 0 \quad (8)$$

Since the value of  $\text{Br}_2$  is small the equation was solved easily by the method of successive approximations. In a preliminary calculation  $\text{Br}_2^3$  was neglected and an approximate value of  $\text{Br}_2$  was obtained. The cube of the value thus obtained was then substituted in equation (8) and the resulting quadratic equation solved again for  $\text{Br}_2$ . After repeating this process several times the numerical value of  $\text{Br}_2$  approached a constant which is the correct root of the equation.

The concentration of  $\text{HBrO}$  was calculated from the free bromine ( $\text{Br}_2$ ), total bromide ( $B$ ), and bromine ( $C$ ) by means of equation (9) which was derived by substituting in equation (7) the value of  $\overline{\text{Br}}_3$  from equation (2) and the value of  $\overline{\text{Br}}$  in terms of  $\overline{\text{H}}$ ,  $\text{HBrO}$ , and  $\text{Br}_2$  obtained from equation (1). The numerical value of  $\overline{\text{H}}$  is  $4 \times 10^{-6}$ .

$$\text{HBrO} = \frac{28 \times 10^{-4} (0.102 + \text{Br}_2) (\text{Br}_2)}{2B - C + \text{Br}_2} \quad (9)$$

TABLE 6.—Correlation of rate of oxidation of  $\beta$ -d-glucose with concentration of free bromine<sup>1</sup>

Experiment No.	$\beta$ -d-glucose	$B^2$ (Bromide)	$C^3$ (Bromine)	Free bromine (calculated)	HBrO (calculated)	Oxidation after—			$ak\beta$	$k\beta$
						5 min-utes	10 min-utes	15 min-utes		
						6				
1	2	3	4	5	6			7	8	
	Moles per l	Equiva- lents per l	Moles per l	Moles per l	Moles per l $\times 10^{-4}$	Per cent	Per cent	Per cent		
1-----	0.0496	0.412	0.351	0.0692	0.612	62.5	86.7	95.0	0.0873	1.26
2-----	.0496	.238	.179	.0477	.580	56.7	80.9	91.0	.0714	1.50
3-----	.0496	.564	.186	.0196	.069	26.1	47.4	61.5	.0272	1.39
4-----	.0496	.564	.182	.0192	.068	26.8	47.0	60.5	.0272	1.42
5-----	.0496	.560	.176	.0182	.064	24.7	44.2	59.3	.0253	1.39
Average-----										1.39

<sup>1</sup> The results for each experiment were derived from an experiment similar to that given in Table 3. The temperatures were  $0.5^\circ \text{C.} \pm 0.3^\circ$ , except for experiment 1, which was  $0.3^\circ \text{C.} \pm 0.3^\circ$ . The calculations are based on that data given in this table except for experiment 1 which gives the averages reported in Table 3.

<sup>2</sup> Average bromide content, which corresponds to  $B$  in equation (4), p. 347.

<sup>3</sup> Average bromine by  $\text{Na}_2\text{S}_2\text{O}_3$  titration, which corresponds to  $C$  in equation (5), p. 347.

The experiments tabulated in Table 6 were conducted to give information concerning the active oxidant. The first experiment is the one illustrated in Figure 2, curve 1, and given in detail in Table 3, while the others differ in the amounts of bromine and barium bromide employed. In experiments 2 and 5 approximately equal amounts of bromine were used, yet the values obtained for  $ak_\beta$  (column 7) were 0.0714 and 0.0253, which clearly shows that the reaction rate is not determined by the total quantity of bromine. It is also apparent that hypobromous acid is not the oxidant since a tenfold variation in the  $\text{HBrO}$  was accompanied by only a threefold change in reaction rate. The values for  $k_\beta$ , reported in column 8, based on the assumption that free bromine is the active oxidant, differ slightly among themselves, but they are sufficiently constant to indicate that free bromine is the oxidant. The variations are due in part to small unavoidable differences in temperature. In addition, it will be recalled that the data used for estimating the concentration of free bromine were assumed to be the same as those found by Jones and Hartmann for relatively dilute bromine-potassium bromide solutions and hence the differences in the constants may be partially ascribed to inadequate knowledge as to the concentration of the free bromine in the specific solutions used in these experiments.

Since the oxidation of  $\alpha$ -*D*-glucose is relatively slow, allowance must be made for the stereoisomeric conversion of the alpha to the beta form while the oxidation is taking place. The resulting beta sugar would be oxidized nearly as rapidly as it is formed. Hence, we can consider the oxidation as consisting of two reactions, namely, the oxidation of the alpha form directly, and the conversion of the alpha to the beta form with the subsequent rapid oxidation thereof. In the event that a constant amount of the oxidant is maintained, we can assume as an approximation that we are dealing with only two simultaneous first-order reactions and apply the customary formula to obtain the velocity constants:  $k + k_1 = \frac{1}{t} \log \frac{A}{A-x}$ . In this formula,  $k$  represents the velocity constant for the oxidation of the alpha form directly;  $k_1$  represents the velocity constant for the conversion of the alpha form to the beta with the subsequent oxidation of the same. Inasmuch as the oxidation of the beta sugar is rapid, it can be assumed that the value of  $k_1$  is determined by the rate at which the beta sugar is formed by the mutarotation reaction.

This was calculated from mutarotation data, determined under conditions similar to those used in the oxidation experiments. The value thus obtained, 0.00052, is approximately 20 per cent of the value of the constant representing the sum total of the reaction given in column 5 of Table 4. In other words, the sugar is oxidized about five times as fast as the alpha form of the sugar is converted to the beta form. The amount of alpha glucose converted to beta glucose while the reaction is taking place, is represented in Figure 2, page 346, by curve 4. Since the beta sugar is oxidized nearly as rapidly as it is formed, the distance from the base line to the lower curve represents approximately the amount of alpha glucose which is converted to beta glucose and then oxidized, while the vertical distance from curve 4 to curve 3 represents the maximum amount of alpha glucose oxidized as such. The amount of beta glucose converted to alpha glucose during the oxidation is negligible because the beta form is oxidized so rapidly.



In order to estimate the approximate value for the constant representing the oxidation of alpha glucose directly, it was assumed that the value of  $k_1$  is equal to the constant representing the conversion of alpha glucose to beta glucose. By substituting the value of  $k_1$  determined from mutarotation data, the following equation is obtained

$$k + 0.00052 = \frac{1}{t} \log \frac{A}{A-x}.$$

The value of  $k$  is based on the assumption that a constant amount of the oxidant was present at all times. If this amount be  $a$ , then  $k = ak_\alpha$ , where  $k_\alpha$  is the constant for the oxidation of the alpha form of the sugar in the presence of a molar solution of the active oxidant.

TABLE 7.—Correlation of rate of oxidation of  $\alpha$ -D-glucose with concentration of free bromine<sup>1</sup>

Experiment No.	$\alpha$ -D-glucose	$B^2$ (Bromide)	$C^3$ (Bromine)	Free bromine (calculated)	HBrO (calculated)	Oxidation after—			$k_1 + ak_\alpha$	$ak_\alpha$	$k_\alpha$
						30 minutes	60 minutes	120 minutes			
	1	2	3	4	5	6			7	8	9
	Moles per l	Equivalents per l	Moles per l	Moles per l	Moles per l $\times 10^{-4}$	Per cent	Per cent	Per cent			
1-----	0.0496	0.555	0.56	0.101	0.88	18.9	33.1	52.2	0.00287	0.00235	0.023
2-----	.0496	.381	.374	.085	.94	16.6	28.8	49.1	.00254	.00202	.024
3-----	.0496	.195	.19	.063	1.11	15.2	27.5	46.0	.00231	.00179	.028
4-----	.0496	.550	.365	.061	.28	12.9	25.8	39.8	.00200	.00148	.029
5-----	.0496	.544	.19	.021	.08	5.8	15.3	30.6	.00113	.00061	.029
6-----	.0985	.390	.37	.081	.85	15.4	29.0	45.4	.00236	.00184	.023
Average-----											.026

<sup>1</sup> The results for each experiment were derived from an experiment similar to that given in Table 4. The temperatures were  $0.4^\circ \text{C} \pm 0.2^\circ$ , except for experiment 2, which was  $0.3^\circ \text{C} \pm 0.3^\circ$ . The calculations are based on the data given in this table except for experiment 2 which gives the averages reported in Table 4.

<sup>2</sup> Average bromide content, which corresponds to  $B$  in equation (4).

<sup>3</sup> Average bromine by  $\text{Na}_2\text{S}_2\text{O}_3$  titration.

A series of results obtained by the oxidation of  $\alpha$ -D-glucose with varying amounts of bromine and barium bromide is given in Table 7. The values representing the velocity constants for the total reaction are recorded in column 7 and the velocity constants representing the oxidation of the alpha sugar only are given in columns 8 and 9. The concentration of free bromine was varied by using different amounts of bromine in some cases, and in others different amounts of barium bromide. The maximum concentration of free bromine was 0.101 moles per liter, while the minimum was 0.021, and the corresponding constants ( $k_\alpha$ ) for the oxidation of alpha glucose were 0.023 and 0.029. The values of  $k_\alpha$  are sufficiently uniform to show that the reaction rate is determined largely by the concentration of free bromine, and that the assumption that the reaction consists of two simultaneous reactions was justified.

In some of the oxidations reported in Table 7 there were small undetermined variations in the amount of bromine. In the experiments reported in Table 4 the concentration of bromine was determined at frequent intervals, and hence the velocity constants reported in Table 4 are better values than those of Table 7.

The value of  $(k_1 + ak_\alpha)$  under the conditions outlined in Table 4 is 0.00254, while the constant ( $k_\alpha$ ), representing the oxidation of  $\alpha$ -*d*-glucose only is 0.0238. The ratio of  $(k_1 + ak_\alpha)$  to  $ak_\beta$  depends in part upon the value of the concentration of free bromine,  $a$ . Under the conditions of the experiments given in Tables 3 and 4,  $(k_1 + ak_\alpha):ak_\beta::1:34$ , while  $k_\alpha:k_\beta::1:53$ . In other words, the oxidation of the beta sugar under these specific conditions is 34 times as rapid as the total oxidation of the alpha sugar. If allowance is made for the mutarotation reaction and a small difference in the concentration of free bromine, the oxidation of the beta form is 53 times as fast as the oxidation of the alpha form.

It has been shown that  $\beta$ -*d*-glucose is oxidized rapidly, while  $\alpha$ -*d*-glucose is oxidized much more slowly; the marked difference in the reaction rates will now be used for ascertaining the approximate amounts of each in the equilibrium solution. The oxidation of a sugar solution containing a single isomer usually progresses uniformly, while the oxidation of a mixture of the alpha and beta forms progresses

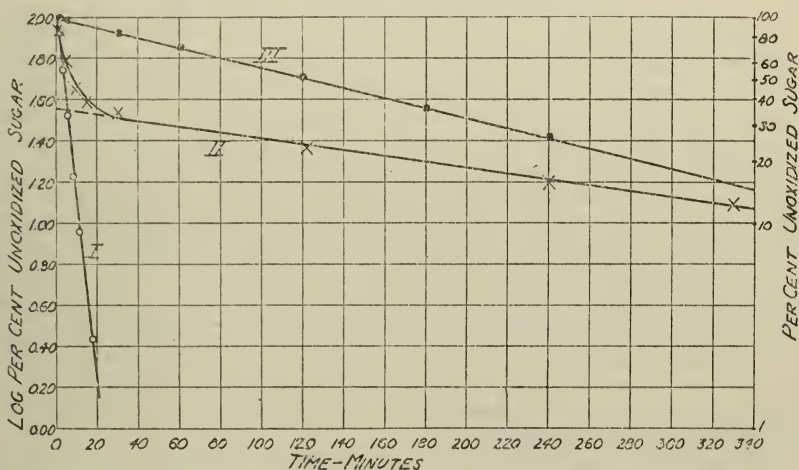


FIGURE 3.—Bromine oxidation of *d*-glucose in the presence of  $\text{BaCO}_3$

rapidly until the beta is used up, and then more slowly as the oxidation of the other form is continued. Although the velocity constants for the reaction at various intervals reveal the changes in the reaction rate, the deviations are more easily detected graphically. Thus, if a pure alpha or beta sugar is oxidized with a large excess (or constant amount) of bromine and the logarithm of the per cent of unoxidized sugar is plotted against time, usually a straight line is obtained which passes through the point representing the logarithm of the initial concentration of the sugar.

This is illustrated in Curves I and III of Figure 3, which were plotted from data obtained from  $\alpha$ -*d* and  $\beta$ -*d*-glucose, respectively. The slope of the curves is a measure of the reaction rate. Note that the curves are approximately linear and that they pass through the point representing the amount of the sample used. This shows that the oxidation progressed at a uniform rate throughout the experiment, which proves that the samples were homogeneous. In marked contrast,



the oxidation of an equilibrium solution proceeds rapidly for the first 15 or 20 minutes and then more slowly. This is illustrated in Curve II of Figure 3, which gives the oxidation of an equilibrium solution of *d*-glucose. The slope of the curve increases during the first 30 minutes after which the curve becomes approximately linear. The latter part of the curve represents the oxidation of  $\alpha$ -*d*-glucose. By extending this curve to zero time the amount of  $\alpha$ -*d*-glucose in the solution at beginning of the reaction can be estimated. The extrapolated curve meets the axis at a point equivalent to 36 per cent. Thus, from the rates of oxidation only, it is found that an equilibrium solution of glucose contains approximately 64 per cent beta and 36 per cent alpha glucose. These values are in qualitative agreement with the values (65 and 35, respectively) determined from the optical rotation of the equilibrium solution. This is additional evidence that an equilibrium solution of glucose consists largely of a mixture of the normal alpha and beta forms of the sugar.

#### IV. EXPERIMENTAL DETAILS

##### 1. OXIDATION OF $\beta$ -*d*-GLUCOSE TO GLUCONIC DELTA LACTONE

Thirty grams of finely powdered barium carbonate was mixed with 200 ml of water in a 500 ml glass-stoppered flask. After saturating the solution with carbon dioxide and adding 10 ml of bromine, 9 g of crystalline  $\beta$ -*d*-glucose was added. The mixture was shaken continuously while the temperature was maintained at 25° C. for three minutes. The flask was then cooled in ice water for one minute, and the reaction stopped by the addition of 40 ml of linseed oil dissolved in 100 ml of cold benzene. The excess bromine was removed in about 15 seconds. The oxidation mixture, containing the benzene solution of linseed oil, was cooled in ice water for about one minute more and then transferred to a 500 ml separatory funnel. The aqueous solution was separated and filtered. The filtrate thus obtained was used for measuring (1) amount of oxidation, (2) the specific rotations, (3) the hydrogen-ion concentrations, (4) the acid formed by hydrolysis, and (5) the rate of saponification.

The amount of oxidation was determined by boiling a 10 ml sample of the solution containing the oxidation product with Benedict's copper sulphate reagent. The cuprous oxide was estimated by Scales' method<sup>21</sup> modified by changing the time of boiling from three to six minutes. The solutions were standardized with solutions of dextrose containing like concentrations of barium bromide. The results showed that the solution of the oxidation product contained 1.07 mg/ml of reducing substance calculated as dextrose. This is equivalent to 2.5 per cent; that is, the oxidation of the sugar was 97.5 per cent complete.

In order to prove that the product of the oxidation consists almost exclusively of the delta lactone, a sample of delta lactone was studied under conditions as nearly like the conditions of the oxidation experiments as possible. The sample of the delta gluconic lactone (8.9 g) was added to a solution of barium bromide (16.46 g of  $\text{BaBr}_2 \cdot 2\text{H}_2\text{O}$ ) in 200 ml of water containing 30 g barium carbonate in suspension and saturated with carbon dioxide. The solution was shaken three minutes at 25° C., cooled, and filtered, and the filtrate used for comparison with the oxidation products.

<sup>21</sup> Scales, J. Ind. Eng. Chem., vol. 11, p. 747, 1919.

The specific rotations of the oxidation products and of the solution containing the delta lactone were measured in a 2 dm water-jacketed tube, with a Bates saccharimeter. The values given in Table 1, page 339, were calculated from the following formula:

$$[\alpha]_D^{20} = \frac{^{\circ}S \times 0.3462 \times 207.8}{2 \times 8.9}$$

The weight of the lactone in each case was 8.9 g and the volume of the solution 207.8 ml. This volume was determined by measuring the volume of a solution containing 8.9 g of delta lactone, 16.46 g of barium bromide, and 200 ml of water. The results are given in Table 1.

Hydrogen-ion concentrations of the solutions containing the delta lactone and the solutions containing the oxidation products were measured at 25° C. with a quinhydrone electrode in the usual manner. The results are given in Table 1.

The acid formed by hydrolysis was determined in the following manner: A 10 ml sample of the oxidation solution was diluted with an equal quantity of water and heated to boiling to remove the dissolved carbon dioxide. The solution was cooled to room temperature and 25 ml of *N*/10 barium hydroxide was added. After five minutes the excess barium hydroxide was titrated with *N*/10 sulphuric acid, using phenolphthalein as an indicator. The 10 ml sample of the oxidation product neutralized 22.47 ml of *N*/10 alkali. The total volume of the solution was 207.8 ml, hence there was 0.0467 mole of lactone present. A 10 ml sample of the solution of the delta lactone which was shaken with barium carbonate and treated in manner analogous to the oxidation product required 22.48 ml *N*/10 alkali for neutralization. The oxidation product required practically the same amount of alkali for neutralization as the sample of the lactone. The agreement shows that the sugar was converted nearly quantitatively into the lactone.

The rates of saponification were determined at various times by measuring the unchanged lactone which remained after shaking the solution with barium carbonate in the presence of carbon dioxide. From time to time samples were taken; the excess barium carbonate was removed by filtration and the filtrate freed from CO<sub>2</sub> by boiling. Then an excess of *N*/10 barium hydroxide was added and the amount of alkali used in saponifying the lactone was determined by back titration with *N*/10 acid. The results, which are given in Table 2 and illustrated in Figure 1, show that the oxidation products derived from  $\alpha$ - and  $\beta$ -*D*-glucose and the equilibrium solution are saponified at approximately the same rate as the delta lactone. The sample of the oxidation product from beta glucose, after 24 hours, required only three drops of *N*/10 alkali to produce a permanent pink coloration with phenolphthalein, while one drop of *N*/10 alkali was sufficient for the sample of the solution of the delta lactone.

As additional evidence for the presence of the delta lactone in the oxidation product, the lactone was separated as soon as possible from the solution obtained by the oxidation of  $\beta$ -*D*-glucose. The experimental conditions were such that the crystalline product could not have been formed from the acid by spontaneous lactone formation. The oxidation was conducted in the following manner: About 15 g of finely powdered barium carbonate was mixed with 9 g of crystalline

$\beta$ -*d*-glucose. The dry mixture was added in portions while stirring to a mixture of 5 ml of bromine and 20 ml of ice water. The reaction was allowed to continue for five minutes, then 10 ml of amylene dissolved in 50 ml of cold dioxane was added and the solution shaken until all of the bromine had combined with the amylene. The dioxane solution, which contained a portion of the product, was decanted from the aqueous sirup which had separated. The aqueous residue was extracted several times with fresh portions of dioxane. The lactone is difficultly soluble in dioxane and care must be taken to secure complete extraction. After filtration a few drops of the dioxane solution on evaporation gave crystalline gluconic delta lactone. This product was separated within one-half hour after the beginning of the experiment. The remaining dioxane solution was evaporated quickly in high vacuo to a sirup. The sirup was transferred to a small breaker and the solution saturated with anhydrous ether and seeded with some of the delta lactone which was obtained as noted above; crystallization began immediately. After one hour the product was separated and dried. About 5 g of nearly pure gluconic delta lactone was obtained. After recrystallization the product melted at 152° C., and 2.5 minutes after solution gave  $[\alpha]_D^{20} = +65.7^\circ$ , which decreased in 400 minutes to  $+7.0^\circ$  (0.9654 g dissolved in 25 ml).

## 2. OXIDATION OF $\alpha$ -*d*-GLUCOSE TO GLUCONIC DELTA LACTONE

A sample of  $\alpha$ -*d*-glucose was oxidized in manner analogous to that given for  $\beta$ -*d*-glucose, except that the oxidation was allowed to continue for 20 minutes at 25° C. rather than for 3 minutes. The oxidation was 85.5 per cent complete. The specific rotations and hydrogen-ion concentration are given in Table 1, page 339. The values obtained for the amount of acid formed by the hydrolysis of the oxidation product and the rate of saponification are of particular interest. A 10 ml sample of the solution of the oxidation product required for neutralization 18.0 ml of *N*/10 alkali; this is equivalent to 0.0374 moles of lactone in the product. Since the oxidation was 85.5 per cent complete, the 9 g sample of dextrose should have given 0.04275 moles of lactone. But as the reaction required 20 minutes and 5 additional minutes were consumed in removing the excess bromine and barium carbonate, some hydrolysis occurred and consequently the amount of lactone in the sample was slightly less than the amount formed by oxidation. The amount of the lactone hydrolyzed during the preparation of the sample can be estimated approximately from the rate of hydrolysis of the delta lactone. If the lactone were exposed to the hydrolytic action of the aqueous barium carbonate 15 minutes on the average, then it would be hydrolyzed approximately 10 per cent and the amount of lactone present in the sample would correspond to approximately 90 per cent of that formed by oxidation. From the foregoing the total lactone formed by oxidation is calculated as  $\frac{0.0374}{0.90} = 0.0416$  mole, which is only slightly less than 0.0427, the

amount which should have been formed by the oxidation. As the amount of hydrolysis can not be accurately estimated, the difference in the two values can not be ascribed, at present, to the formation of some free acid by the oxidation of the sugar. Although it is possible



that a very small portion of the sample of  $\alpha$ -*D*-glucose, or a form of the sugar derived therefrom, is oxidized by bromine water in the presence of barium carbonate to give the free acid, the results clearly show that the product of the reaction consists for the most part of the delta lactone.

### 3. MEASUREMENT OF REACTION RATES

The oxidations were conducted in a 1-liter, 3-neck flask equipped with a mechanical stirrer. The stirrer was inserted in the center neck, while a tube for admitting carbon dioxide was inserted in one of the outer necks. The third neck was used for introducing the sugar sample and for withdrawing samples of the reaction mixture. At other times it was kept closed with a stopper carrying a thermometer. The reaction flask was placed in an ice bath and the solution in the flask and the ice water were stirred continuously. The solution of the salt containing 15, 30, or 45 g of  $\text{BaBr}_2 \cdot 2\text{H}_2\text{O}$  in sufficient water to make 500 ml and 30 g of barium carbonate was saturated with carbon dioxide and 5, 10, or 15 ml of bromine was added. After mixing for a few minutes 4.5 g of the crystalline sugar was introduced through one of the outer necks in the flask. The mixture was stirred continuously and carbon dioxide, previously bubbled through bromine water, was slowly passed through the solution. Time was measured from the moment that the sugar was added. In the oxidation of the equilibrium solution a portion of the water ordinarily used for preparing the bromide-bromine solution was used for dissolving the sugar. The sugar solution in equilibrium was then added in place of the crystalline sugar. Samples of the reaction mixture were withdrawn at various times and the oxidations of these were stopped quickly by the addition of about 10 ml of linseed oil dissolved in 20 ml of benzene. The amount of unoxidized sugar was determined by reduction of Benedict's copper sulphate reagent. The total bromine was determined by titration of samples of the oxidation mixtures with standard sodium thiosulphate in the usual manner.

The total bromide at any moment is equal to that added plus two equivalents for each mole of sugar oxidized. The average bromide content during the reaction ( $B$  in equation (4)) was calculated from the mean amounts of bromide present between observations, multiplying each mean by the time interval for which it was the average, and dividing the sum of the products by the total time covered. The bromide content ( $B$ ) which is expressed in equivalents of bromide is different from the bromide concentration ( $\overline{\text{Br}}$ ) which represents the actual concentration of the bromide ion or molecule. The volume at the beginning of the reaction was determined experimentally, and the volume at the end was estimated from the increase in the volume of a similar solution upon the addition of the amount of barium bromide formed in the reaction. It was assumed that the variation at intermediate points was proportional to the amount of oxidation.

The results are given in Tables 3, 4, 5, 6, and 7. The velocity constants are based on the amount of sugar as determined by analysis, taking into account the change in the volume of the solution. The first experimental determination was taken as the starting point because in this way errors incident to the preparation of the solution and to the analysis are largely eliminated.

4. MEASUREMENT OF MUTAROTATION OF  $\alpha$ -D-GLUCOSE AT 0° C.

A pipette holding 38.53 g of water at 0° C. was emptied into a flask in which 1.6832 g of  $\alpha$ -D-glucose had been placed. When all the sugar had dissolved, a 2 dm water-jacketed tube, which had been previously cooled to about 0° C., was filled with the solution. The rotations observed with a Bates saccharimeter at various times after solution were: 27.10° S. (4 minutes); 27.06° S. (6 minutes); 26.71° S. (19 minutes); 26.13° S. (40.5 minutes); 24.40° S. (121 minutes); and 22.09° S. (240 minutes). Twenty-four hours later the solution gave a constant reading of 12.88° S. The temperature of the solution was  $1.5^\circ \pm 0.5^\circ$ . The density as determined at 2° C. with a picnometer was 1.0176 g/ml.

The velocity constants were calculated by the usual formula:

$$(k_1 + k_2) = \frac{1}{t} \log \frac{r_0 - r_\infty}{r_t - r_\infty}$$

The average value so obtained for  $(k_1 + k_2)$  was 0.00080. The amounts of alpha and beta glucose in the equilibrium solution were calculated from the specific rotations of alpha and beta glucose and of the equilibrium solution. The values used<sup>22</sup> for these quantities were  $[\alpha]_D^{20} = +113.4^\circ$ ,  $+19.7^\circ$ , and  $+52.2^\circ$ , respectively. These values give  $\frac{k_1}{k_2} = 1.883$ , from which  $k_1$  was determined as 0.00052 at 1.5° C. All logarithms in the paper are expressed to the base.

## V. APPLICATION OF THE METHOD

It is believed that the experimental methods which are described in this and the previous publications can be used for studying the various active forms of the sugars in solution and for determining some of the pertinent questions relating to the composition of sugar solutions. For instance, it has been shown previously<sup>23</sup> that the equilibrium between the various forms of D-glucose may be varied by the addition of calcium chloride. Such changes in the equilibrium may be followed by measuring the reaction rate. Also the rotation of the at present unknown  $\beta$ -D-glucose may be estimated from the composition of the solutions, together with the optical rotation of the alpha form. The oxidation method is being applied also to the products obtained from the various saccharides by enzyme hydrolysis to ascertain the substances formed. Oxidations are being conducted on the alpha and beta forms of the principal sugars in order to determine whether their solutions react solely as mixtures of the alpha and beta forms, or whether the oxidations reveal the presence of one or more additional substances. These studies are being made under strictly comparable conditions so that they will form an adequate basis for the comparison of the reactivity of the various isomeric forms of different sugars.

WASHINGTON, January 11, 1933.

<sup>22</sup> International Critical Tables, vol. 2, p. 347.

<sup>23</sup> Isbell, B. S. Jour. Research, vol. 5 (RP226), p. 741, 1930.











